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The human LINE-1 retrotransposon (L1Hs) is known to cause mutations by inserting into genes and inactivating them. The element is expressed in many breast tumors and breast tumor cell lines, suggesting that L1Hs-induced mutations may play some role in this malignancy. Prior evidence suggested that unmethylated L1Hs elements are transcriptionally active. Therefore, we used the technique of inverse polymerase chain reaction to clone twelve elements that are unmethylated in the breast tumor cell line, T47D. A preliminary characterization has shown that some of these elements are unmethylated in other breast cancer cell lines, but are methylated in a normal breast cell line that does not express L1Hs. These elements are also methylated in several germ cell tumor lines that express L1Hs. Our results to date suggest the possibility that different subsets of active L1Hs elements are expressed in different cancers.

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Jan Fanning March 10, 1994
PI - Signature Date

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Introduction

Human LINE-1 (L1Hs) is a transposable element that encodes a reverse transcriptase and moves via an RNA intermediate [1]. It therefore seems possible that cells in which L1Hs is active may be subject to insertional mutagenesis. We have recently found that the element is expressed in a significant number of germ cell cancers [2,3] as well as in many breast tumors and breast tumor cell lines [4]. This last finding raises the possibility that the initiation or progression of some breast cancers is facilitated by L1Hs-induced insertional mutations.

In addition to insertional mutagenesis, there are several other characteristics of the L1Hs element that suggest its potential as an oncogenic agent. For example, L1Hs has an internal promoter which could potentially lead to readthrough transcription and activation of downstream genes. In addition, the L1Hs-encoded p40 protein has a leucine zipper motif, suggesting a possible interaction with other cellular proteins. Such interactions at inappropriate times might lead to the disruption of important cellular functions. Thus, L1Hs involvement in cancer could occur by several mechanisms, either singly or in combination.

Our long range goals focus on the identification and characterization of L1Hs elements that are expressed in breast cancers. L1Hs expression may identify a class of breast neoplasms in which L1Hs-encoded proteins initiate or maintain the neoplastic state. This suggests that down-regulating L1Hs expression could influence the course of breast cancer. To approach this problem we have proposed to study the expression of L1Hs in cell lines and in solid tumors by cloning and characterizing actively expressed L1Hs elements. Specifically we have proposed to:

(1) Clone active L1Hs elements from several breast cancer cell lines.

(2) Characterize the active L1Hs sequences and determine if the same L1Hs elements are active in solid tumors.

Experimental

Our original plan was to isolate unmethylated (potentially active) L1Hs elements by the method of inverse polymerase chain reaction (PCR) [5], and this approach seems to be working quite well. Our current protocol runs as follows: we partially cleave DNA extracted from the T47D breast tumor line with HpaII, an enzyme that appear to cleave L1Hs sequences only in transcriptionally active cells [6]. We then isolate fragments from 0.6-2.5 kb in size on acrylamide gels and circularize the fragments by incubation with ligase under conditions of dilute DNA concentration (1-2 ng/ul). We collect the circularized DNAs and cleave with the restriction enzyme BglI (BglI has a consensus site located at position 10-20 on the L1Hs consensus sequence; the enzyme introduces cuts between nucleotides 13/14 and 16/17 giving 3' overhangs) and perform PCR under relaxed conditions with primers that are complementary to regions 3-16 and 14-27 on the L1Hs consensus sequence. This results in PCR products that contain 5' flanking DNA bracketed by L1Hs sequences. In most cases the L1Hs moiety of the clone stretches from bp 1 to a HpaII site at bp 36.

Using this technique we have isolated over a dozen interesting clones from the T47D breast tumor line and have partially characterized three of them. All three clones are unmethylated in the T47D cell line, and they are unmethylated in other breast tumor cell lines (e.g., SKBR-3) as well. The clones tested so far are completely methylated in two L1Hs-expressing germ cell tumor lines (NTera2D1 and 2102Ep) and two of the three are

methylated in the "normal" breast epithelial line, HBL100, that does not express L1Hs. In addition, one of the clones is amplified about 10-20-fold in the SKBR-3 breast tumor line, but not in any of the other cell lines. We are currently characterizing the remaining clones as to their methylation status in other tumor cell lines. These include breast, germ cell, and brain tumor lines that we have already shown are actively expressing L1Hs [6].

The most interesting clone we have found to date is designated 593F. The L1Hs-593F element appears to be located within a region of the genome on chromosome 19 that codes for the carcinoembryonic antigen (CEA)/pregnancy-specific glycoprotein (PSG) multigene family. The CEA/PSG region is coordinately expressed in a number of epithelial cancers. This suggested to us that the expression of genes within the CEA/PSG region might also involve L1Hs-593F. Indeed, when we examined the recently characterized promoter for the PSG11 gene [7], we discovered that some PSG11 promoter motifs are present in the 5' ends of L1Hs elements. We are currently examining whether these motifs are components of the L1Hs promoter by making expression constructs that contain the 5' end of L1Hs with mutations in these motifs.

The above results strongly suggest that the original plan to isolate active L1Hs elements from breast tumor cells is viable. We have isolated a number of elements that have all of the characteristics of elements that are expected to be active. By a stroke of luck we have also been able to provisionally map one of the elements to the CEA/PSG region of the genome. This suggests a model for transcriptional activation of at least some L1Hs elements: during tumor initiation or progression certain regions of the genome become transcriptionally active for reasons that are not yet known. Transcriptionally competent L1Hs elements within these regions are also expressed, and their products play an as yet unknown role in malignancy. We are currently testing the validity of this model using other L1Hs clones and tumor cell lines.

Conclusions

We have partially characterized a number of L1Hs elements that may be active in the T47D breast cancer cell line. Preliminary data suggests that at least some of these elements may neighbor other genes that are known to be expressed in malignant cells. This leads to the interesting possibility that unmethylated L1Hs clones may be used to identify and isolate neighboring genes that become transcriptionally active in breast cancer. Some of these genes may also play a role in this cancer.

We have preliminary information that L1Hs elements that are unmethylated in breast tumors are methylated in germ cell tumors. This suggests that specific, non-overlapping sets of elements are active in different cancers. We are currently testing this by isolating unmethylated L1Hs elements from an embryonal carcinoma cell line and from a medulloblastoma cell line. If there are cancer-specific patterns of L1Hs expression, the probes we are developing today may be useful as clinical tools in the future.

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